Available online at www.sciencedirect.com
SCIENCE DIRECT®
Environmental Pollution 125 (2003) 61–70
ENVIRONMENTAL POLLUTION
www.elsevier.com/locate/envpol

Interpreting spatial variation in ozone symptoms shown by cutleaf cone flower, *Rudbeckia laciniata* L.

A.W. Davison[a,*], H.S. Neufeld[b], A.H. Chappelka[c], Kirsten Wolff[a], P.L. Finkelstein[d]

[a]*School of Biology, Ridley Building, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK*
[b]*Department of Biology, 572, Rivers St., Appalachian State University, Boone, NC 28608, USA*
[c]*School of Forestry & Wildlife Sciences, 108 M. White-Smith Hall, Auburn University, Auburn, AL 36849, USA*
[d]*Atmospheric Modeling Division, EPA, MD-80, Research Triangle Park, NC 27711, USA*

Received 17 June 2002; accepted 31 January 2003

"Capsule": *Within-population variation in ozone injury shown by coneflower (*Rudbeckia laciniata*) is shown to be strongly influenced by micro-environment and it is concluded that light (PAR) may play a key role in producing this variation.*

Abstract

Visible injury caused by ozone is recorded every year in native plant species growing in Great Smoky Mountains National Park (USA). One of the most sensitive species, cutleaf coneflower (*Rudbeckia laciniata* L.), shows great variation in symptoms between and within populations but the causes of this variation and its ecological significance are currently unknown. This paper presents data relating to genetic variation, ozone concentrations, stomatal conductance and light (PAR) within populations. The data show that populations differ in genetic diversity, one consisting of only three genets while another was very diverse. In the former population, symptoms varied greatly within a single genet, pointing to a large micro-environmental influence. Measurements of ozone, stomatal conductance and PAR within plant canopies suggest that variation in symptom expression is unlikely to be due to differences in ozone flux and more likely to be due to variation in light. The variation in visible symptoms raises the question of what bioindicators actually indicate, and it suggests that symptoms should be interpreted with great caution until the underlying causes of that variation are fully understood.

Keywords: Ozone; Visible injury; Bioindicator; Coneflower; *Rudbeckia laciniata*

1. Introduction

Great Smoky Mountains National Park (GSMP) is designated as a Class 1 area where resources are to be protected from damage due to air pollution (Shaver et al., 1994) but visible ozone injury has been recorded for over a decade in native plants growing in the park (Neufeld et al., 1992; Chappelka et al., 1997). In 1995, 95 species were reported as showing symptoms that resembled those caused by ozone (Neufeld et al., 1992). When 28 of the 95 species were fumigated in open top chambers in GSMP, 25 were injured by the fumigation. Cutleaf coneflower, *Rudbeckia laciniata*, was classed as extremely sensitive (Neufeld et al., 1992). The distinctive symptoms of ozone injury shown by this species start as patches of dull-red, mottled areas between the veins of the upper surfaces of the lower leaves. The mottle may develop into tan or brown necrotic patches and the affected leaves senesce prematurely. As the season progresses, the symptoms often spread up the plant to the younger leaves.

There is marked variation in the degree of injury shown by individual plants within populations, and between populations that are relatively close to each other (Chappelka et al., this volume). This pattern is shown by several species that grow in GSMP, notably cutleaf coneflower, crown beard (*Verbesina occidentalis*), black cherry (*Prunus serotina*) and tall milkweed (*Asclepias exaltata*). In the case of coneflower we recorded significant variation in symptoms between

* Corresponding author. Tel.: +44-191-222-7890; fax: +44-191-222-5229.
E-mail address: a.w.davison@ncl.ac.uk (A.W. Davison).

0269-7491/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0269-7491(03)00087-3

populations that are only about 200 m apart, and at Clingman's Dome there was significantly greater injury in coneflower plants that were on the edge of a trail than in plants that were as little as 20–30 cm away, off the trail (Chappelka, unpublished). Clearly, if the ecological significance of ozone injury is to be assessed and if symptoms are to be used in bioindication, the it is essential to know the reasons for this spatial variation.

There are several potential causes of variation in the degree of injury, notably: differences in the genetic composition of populations; variation in the ozone concentration between and within populations, and in ozone flux due to differences in the atmospheric and canopy conductances; the age or stage of development of leaves; and several environmental factors that may influence sensitivity or expression of symptoms. This paper focuses on variation within populations of coneflower. We present data on the genetic composition of populations, and on differences in ozone concentrations, stomatal conductance and light within populations.

It is known that there are heritable differences in ozone sensitivity within native species (Berrang et al., 1986, 1989, 1991; Karnosky et al., 1998; Davison and Reiling, 1995; Whitfield et al., 1997; Wolff et al., 2000) so it is possible that some of the variation observed in GSMP is due to genetic differences in the composition of populations. However, coneflower is rhizomatous and spreads vegetatively so it is also possible that populations may consist of a very small number of genets. This could result in there being little heritable variation in ozone response within populations. In order to investigate this, samples of coneflower from Clingman's Dome and Purchase Knob were DNA fingerprinted. This paper reports the results of the DNA fingerprinting.

Uptake of ozone by leaves and deposition on surfaces reduces the concentration inside canopies. Therefore variation in ozone concentrations due to differences in leaf area index or stomatal conductance may contribute to the difference in injury such as that between on- and off-trail plants at Clingman's Dome. There have been several comprehensive studies of ozone concentrations in tree canopies (Enders, 1992; Fontan et al., 1992; Fredricksen et al., 1995; Joss and Graber, 1996; Lorenzini and Nali, 1995; Neufeld et al., 1992; Samuelson and Kelly, 1997) but at present there are no data on ozone gradients in stands of tall herbaceous species. Therefore, in 2001, ozone profiles were measured in coneflower populations at Clingmans Dome and Purchase Knob.

The flux of ozone into a plant canopy depends on the atmospheric, boundary layer and canopy conductances (Cape and Unsworth, 1988). As air movement is usually reduced inside a dense canopy, the atmospheric and boundary layer conductances are lower and as a consequence uptake of ozone would be expected to be lower than in leaves that are on the outside. However, stomatal conductance is probably the most important control on uptake so we measured conductances at the edges and centres of populations, and compared individual plants that differed in the degree of injury shown by the lower leaves.

In GSMP, coneflower populations range from about a meter to over 30 m across. They are found in open areas and on the fringes of forest stands where they may be shaded for at least part of the day. Several important environmental factors differ between the outside edges of populations and inside, notably vapour pressure deficit (vpd) and light. Many species are sensitive to changes in vpd and it is an important factor in causing variation in ozone injury (Balls et al., 1996). Irradiance and spectral quality also affect stomatal conductance and they vary with canopy height, density and leaf area index so differences in the structural morphology of populations may also be a contributory factor. Differences in the light environment may also be significant because the main symptom shown by coneflower is the production of anthocyanin, which is well known to be influenced by light (Craker and Wetherbee, 1973; Rabino and Mancinelli, 1986; Mancinelli, 1990; Cone et al., 1993). Therefore we recorded vapour pressure deficits and light profiles in populations of coneflower.

2. Materials and methods

2.1. Field sites and ozone symptoms

All field measurements were made at two locations in Great Smoky Mountains National Park: Clingmans Dome (35.562 N, 83.502 W, elevation = 1951 m) on the North Carolina/Tennessee border; and at the Highlands Science Learning Center, Purchase Knob, North Carolina (35.588 N, 83.074 W, elevation 1515 m).

2.2. DNA fingerprinting

In August 2000 and 2001, plants were collected from the field sites for DNA fingerprinting. In 2000 small pieces of rhizome were collected from six plants that exhibited visible foliar ozone symptoms and six that were uninjured. The rhizomes were collected along two 10-m transects, on and off the Clingman's Dome trail (Table 1, population 2). The individual shoots were approximately 50–100 cm apart. The rhizomes were cultivated at Newcastle University and DNA was extracted from young, expanding leaves using a standard CTAB (cetyl trimethyl ammonium bromide) method (Weising et al., 1995). Samples were fingerprinted using RAPD analysis (Weising et al., 1995). Five primers (Operon Technologies Inc, USA) were

Table 1
Characteristics of populations of cutleaf coneflower, *Rudbeckia laciniata*, at two sites in the Great Smoky Mountains National Park, 9 August 2001[a]

Site	Location of population	% of plants with O_3 injury, July 2001	Mean plant height cm	LAI at ground level	LAI at base of 1st flower stems
Clingman's Dome TN. Latitude 35.5628 N Longitude 83.4981 W Elevation ca. 1900 m. Open area under dead Fraser fir (*Abies fraseri*) with red spruce (*Picea rubens*) and mountain ash (*Sorbus americana*)	1. Next to parking lot	Not recorded	132 (2.4)	5.46 (0.11)	0.75 (0.05)
	2a. On edge of paved trail to summit	100	137 (2.4)	4.93 (0.13)	0.59 (0.05)
	2b. Same as 2a but just off-trail	40	171 (6.0)	5.39 (0.31)	0.83 (0.20)
Purchase Knob NC. Latitude 35.59 N Longitude 83.0775 W Elevation 1536 m. Meadow grading into buckeye dominated forest (*Aesculus octandra*) with some cherry, *Prunus serotina*. Elsewhere: *Quercus rubra*, *Q. prinus*, *Betula alleghaniensis* and *Acer rubrum*.	3a. Outside forest, merging into meadow	52	151 (8.1)	3.83 (0.19)	0.59 (0.06)
	3b. Forest shade	28	134 (4.2)	Plant density too low	–

[a] Mean and standard error ($n = 3$–5) of plant height and leaf area index (LAI, dimensionless). Ozone injury (% of plants in 2001), data from Chappelka et al. (this volume).

used. Preliminary work with the plants showed that DNA could be successfully extracted from leaves stored in sealed polythene bags for 4–5 days so in August 2001, young healthy leaves were collected from Clingman's Dome (Table 1, populations 2a and b) and Purchase Knob (Table 1, populations 3a and b), sealed in polythene bags and extracted at Newcastle 3 days later. At each site, a young leaf was collected from an individual that showed severe injury symptoms and from its nearest, uninjured neighbour. Three pairs were collected at about 50–100 cm intervals along transects at Clingman's Dome and 10 pairs at about 5–10 m intervals at Purchase Knob.

2.3. Ozone and light gradients in coneflower populations

Ozone and light gradients were measured in populations at Clingman's Dome and Purchase Knob in July and August 2001. The plants at Clingman's Dome ranged from 132 to 171 cm in height and had high leaf area indices (= LAI, measured with a Li-Cor 2000 Plant Canopy Analyzer) ranging from 4.9 to 5.4. At Purchase Knob plants in full sun were 151 cm high and the population had a lower LAI of 3.8 (Table 1). In the Purchase Knob forest, the mean height was 134 cm. The LAI was too low to measure accurately because of low stem density. The number of leaves per plant in all populations ranged from about 8–13 (Chappelka et al., this volume).

Ozone was measured using three 2B Technologies (Golden, CO) portable ozone monitors run from rechargeable battery packs. The monitors were fitted with 4 m long×6 mm diameter Teflon sampling tubes and a Teflon membrane filter was fitted to the open end of each tube to prevent ingress of particles and debris. At the end of each day monitors were cross-checked by tying the intake ends of the three sample tubes next to each other and logging the ozone concentrations each minute for 20–30 min. Two instruments were within 1 nl O_3 l^{-1} of each other and the other was consistently 1–2 nl O_3 l^{-1} higher. Readings were corrected for this small difference. At Purchase Knob, readings were also checked against a North Carolina State monitoring station instrument that was situated about 40 m from one of the coneflower populations and all three were within 2 nl O_3 l^{-1} of that instrument. Ozone concentrations during the period of measurement in 2001 (24 July–6 August) are given in Chapelka et al. (this volume).

Light gradients were measured using two factory-calibrated Delta-T Devices (Burwell, Cambridge, UK) integrating PAR (photosynthetically active radiation, 400–700 nm) sensors. Profiles were measured by placing the O_3 sample tubes and light sensors above and at various positions in the canopy by means of 1.3 cm diameter wooden poles and lab scaffold. At each population, an ozone sample inlet and light sensor was mounted as near to 1.5× canopy height as possible to act as reference points and a second pair was inserted laterally into the population with minimum disturbance. Readings of ozone and light at both points were logged every minute for periods of at least 20 min then the pair of in-canopy sensors was moved to a different location in the canopy. The third ozone monitor was used to record at the edge of populations for comparison with

the interior, and for measuring the gradient of ozone from the edge to the inside of the forest at Purchase Knob. The ozone and light inside the canopy were expressed as percentages of the values at the reference position. Data are reported for one transect from the edge to the interior of a population and for vertical gradients in five populations (Table 1).

2.4. Stomatal conductance

At the start of each study (2000 and 2001), five individual plants at each site were fitted with numbered tags and the uppermost and lowermost (non-senescent) leaf on each plant was marked using waterproof marker or a coloured tag. The stomatal conductance of the adaxial surfaces of each upper and lower leaf was measured at approximately hourly intervals using a Delta T porometer. The instrument was calibrated at 1–2 h intervals, and cross-checked against a Li-Cor 6200 instrument (Li-Cor Inc., Lincoln, NE, USA) that was being used at the same time to measure net assimilation rates (Neufeld, unpublished). The instruments were cross-checked by recording conductance (mmol m^{-2} s^{-1}) on the same leaves of over 30 species of trees and forbs. The regression was: Li-Cor conductance = exp(0.0024 × Delta T conductance) + 126 ($n = 53$, $r^2 = 0.904$). The relationship was linear between about 120 and 600 mmol: Li-Cor conductance = 0.864 × Delta T conductance + 27 ($n = 34$, $r^2 = 0.871$). Stem xylem water potentials were measured using a Scholander-type pressure vessel.

2.5. Statistical analysis

Statistical comparisons of stomatal conductance were made using *t*-tests and/or analysis of variance (Minitab) where appropriate, and regressions were calculated using Grapher software (Golden Software, Golden Co, USA).

3. Results

3.1. The genetic diversity of coneflower populations

Five primers were used for DNA fingerprinting. Results for the Clingman's Dome plants using primer A12 are shown in Fig. 1 as an example. The first four tracks from the left were plants collected on the trail (population 2a, Table 1). The last six tracks were collected off the trail (population 2b, Table 1). Examination of all five primers indicated that all off-trail plants were the same genet. The on-trail plants consisted of two very similar genets but they were also very similar to the off-trail plants. The degree of similarity could not be assessed any more closely because of the limits of RAPDS.

At Purchase Knob, ten pairs of plants were sampled, five pairs from the forest edge (Population 3a, Table 1) and five from shade (Population 3b, Table 1). In Fig. 2 the first track in each pair is from an individual that was injured and the second is from its nearest non-injured neighbour. The individuals in each pair were from about 20 cm to 1 m apart. The tracks show clearly that all individuals were genetically distinct and that there was great genetic diversity in the forest edge and shade populations.

3.2. Gradients in ozone and light in populations

The gradient in ozone and light from the edge into a small (5 m × 3 m) population (1, Table 1) of coneflower at Clingman's Dome in August 2001 is illustrated in Fig. 3. This population was chosen because it had a very abrupt edge and it was on level ground. All readings were taken with the in-canopy PAR sensor and ozone sample tube held 1.5 cm above the soil. Irradiance dropped rapidly with distance from the edge, reaching 1.5% of full daylight approximately 130 cm from the edge. Ozone fell more gradually to 42% of the reference value at 130 cm. All subsequent vertical profiles were measured with sensors at least 150 cm from the edges of populations.

Vertical profiles of light and ozone were measured in the centre of the same population (Fig. 4). Light was again reduced much more rapidly than ozone. For example, about 50 cm above the soil, the light was less than 5% of that at the reference position but the ozone was still around 90%.

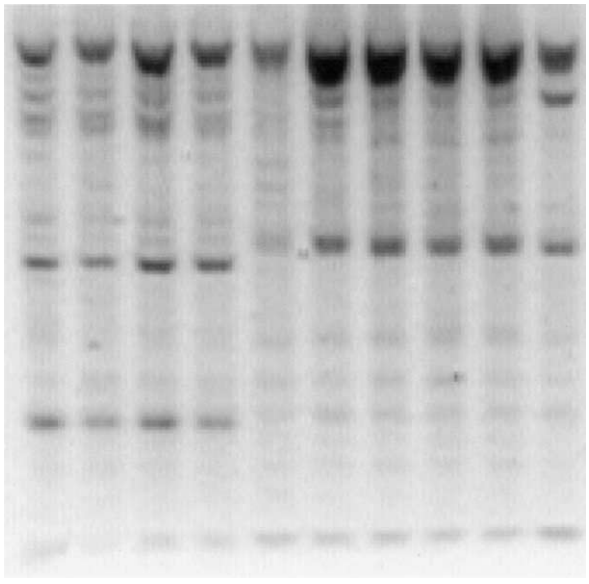

Fig. 1. Example of DNA fingerprints (Operon Technologies primer A12) of 10 plants of coneflower, *Rudbeckia laciniata*, collected from populations 2a and 2b at Clingman's Dome, August 2000. The first four tracks from the left were plants from the population (2a) on-trail. The last six were plants from the population (2b) off-trail.

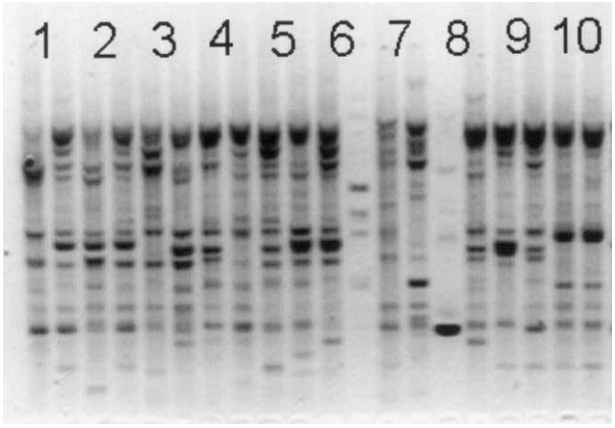

Fig. 2. Example of DNA fingerprints (primer A12) of 10 pairs of plants of coneflower, *Rudbeckia laciniata*, collected at Purchase Knob, August 2001. The first plant in each pair showed ozone injury symptoms and the second was its nearest uninjured neighbour. The first five pairs were from a shaded forest site (population 3b) and the second five from the nearby sunny forest edge (population 3a). All plants showed very different patterns.

Vertical profiles of ozone and PAR were recorded in four other populations (Table 1, populations 2a, 2b, 3a, 3b). The two main features of the data (Fig. 5) are the greater variability in ozone concentrations than in PAR, and the greater reduction in PAR than in ozone. At a height of 50 cm above ground, which included the first four leaves, the ozone varied from about 15–90% of ambient, whereas PAR was consistently $<10\%$. The relationship between ozone and PAR depletion is summarised in Fig. 6, where the ozone depletion = Ozone = 24.5*log10 (PAR depletion) + 43, $r^2 = 0.474$, $n = 25$, $P < 0.02$.

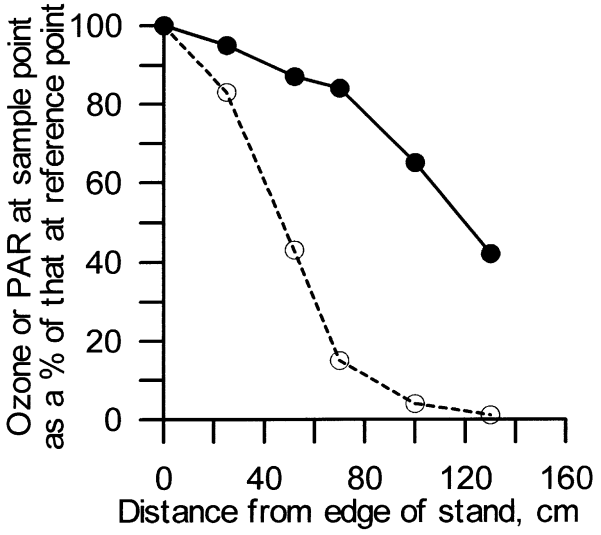

Fig. 3. Change in ozone concentration and PAR with distance from the edge of a population (1, Table 1) of cutleaf coneflower, *Rudbeckia laciniata*, at Clingmans Dome, August 2001. All readings were taken 1.5 cm above soil level and are expressed as a per cent of values at a reference point 1.5× canopy height above the population. ● = ozone, ○ = PAR.

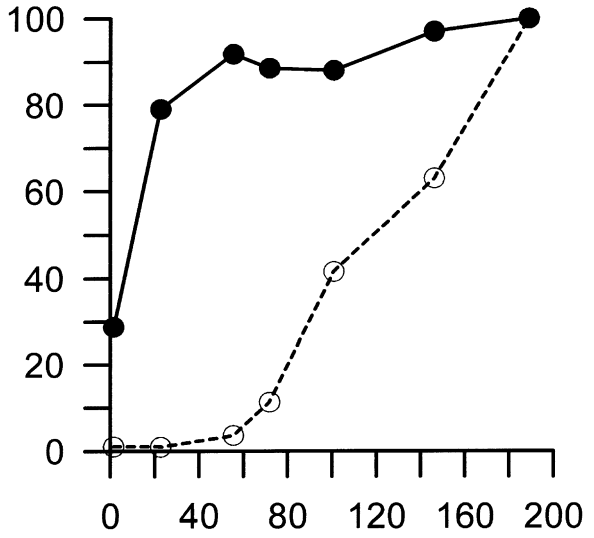

Fig. 4. Vertical profiles of ozone and PAR in the centre of a population (1, Table 1) of cutleaf coneflower at Clingman's Dome, August 2001. All readings are expressed as a per cent of values at a reference point 1.5×canopy height above the population. ● = ozone, ○ = PAR.

3.3. Stomatal conductance

The pattern of stomatal conductance in the on- and-off trail plants at Clingman's Dome (Table 1, populations 2a and 2b) is illustrated in Figs. 7 and 8. The data were recorded on 2 days in July 2000 but similar data were collected in 2001. Fig. 7 shows data collected on a day that was dull and overcast before a rain storm at 14:00 h (24 July). PAR was low both on- and off-trail (Fig. 7b and d) and vpd was relatively constant around 600–800 Pa. On the edge of the trail (Fig. 7a) stomatal conductance was high in the morning, falling to around 200–250 mmol m^{-2} s^{-1} when readings ended at 13:40 h. There was no difference between upper and lower leaves, except for the first reading. Off the trail the pattern of conductance was different (Fig. 7c), with no steady fall and a consistent difference between upper and lower leaves. At the last reading, the conductance off the trail was around 300–400 mmol compared with 150–200 in plants that were on the trail ($P < 0.01$).

In contrast, the data in Fig. 8 were recorded on a day that changed from being initially cloudy to bright sunshine (20 July). PAR rose to almost 1600 μmol m^{-2} s^{-1} at the edge of the trail (Fig. 8b). Humidity was high but the vpd increased from 750 to 1059 Pa at the edge of the trail. The conductance of leaves on the edge of the trail showed the same pattern as in Fig. 7 but around 10:00 h, shortly after the sun hit the upper leaves, they began to wilt. This was reflected in a change in the stem xylem water potentials (Fig. 9). At 12:10 h the conductance of leaves on-trail was down to 60–110 mmol. Off-trail, conductance was lower in the lower leaves but it rose when the irradiance increased. At 12:10 h the conductance off-trail was around 200–260 mmol, significantly ($P < 0.01$)

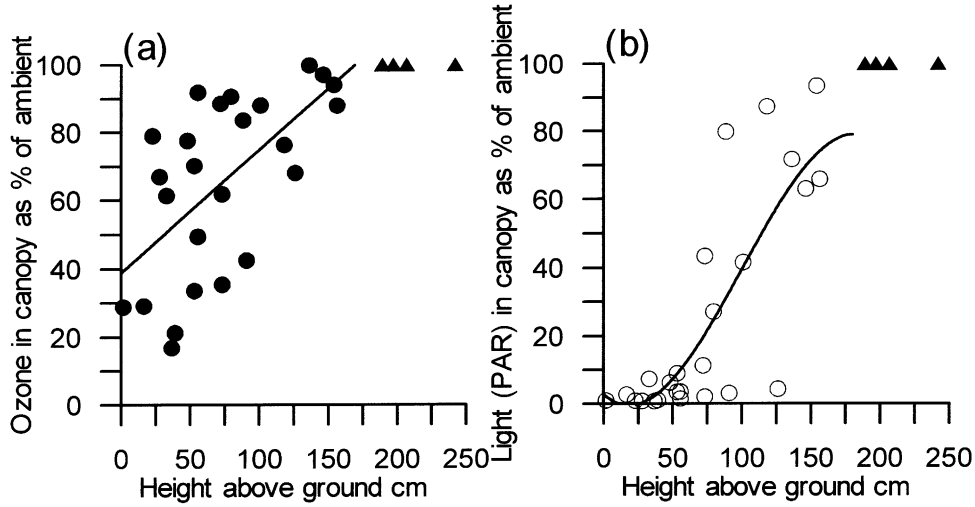

Fig. 5. Composite graphs of (a) four ozone and (b) PAR profiles in populations of cutleaf coneflower at Clingman's Dome and Purchase Knob (populations 2a, 2b, 3a, 3b, Table 1), August 2001. ● = ozone, ○ = PAR, ▲ = ozone and PAR at reference heights above the canopies for the four profiles. Regressions: (a) ozone = 0.36×(height above ground, cm) + 38.8, $r^2 = 0.382$, $n = 25$, $P < 0.002$; (b) PAR = 2.82−0.371×(h, height above ground, cm) + 0.011×h^2−0.000037×h^3; $n = 25$, $r^2 = 0.635$, $P < 0.001$.

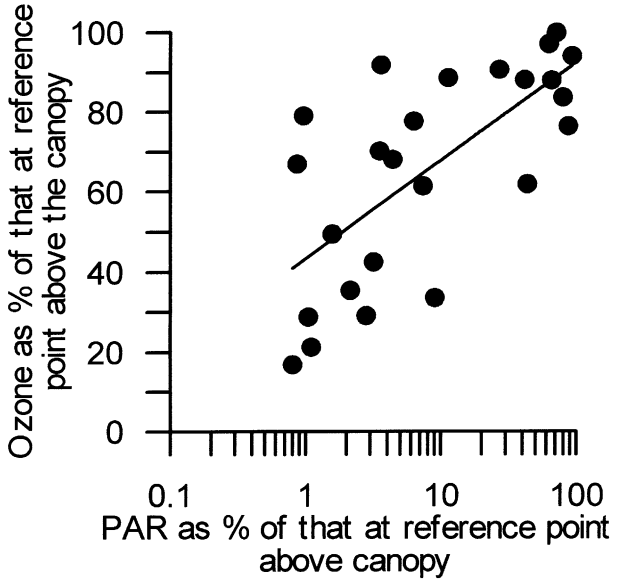

Fig. 6. Summary of the relationship between ozone and PAR in populations of cutleaf coneflower at Clingman's Dome and Purchase Knob, August 2001. Ozone = 24.5×log10(PAR) + 43, $r^2 = 0.474$, $n = 25$, $P < 0.02$.

higher than on-trail. Readings were terminated after 12:10 h due to instrument failure but the data illustrate that on 2 days when conditions were very different, the same patterns of conductance were seen. At some times of day, off-trail plants that showed less ozone injury, had higher conductances than those on-trail.

4. Discussion

Although ozone visible injury is recorded in most industrialised countries and is used as evidence of biological impact, there have been surprisingly few critical attempts to assess the relationships between ozone exposure, visible injury and ecological effects on herbaceous species (Davison and Barnes, 1998). Perhaps one of the most telling studies of visible injury was that by Krupa et al. (1993). They exposed the sensitive Bel W3 tobacco for weekly periods in open top chambers at two locations in the USA. Using a range of ozone descriptors, they were able to produce statistically significant relations between ozone exposure and injury but even the best models accounted for only 30–32% of the variability. They concluded that tobacco varieties can be used as a qualitative, but not necessarily quantitative indicator of relative ozone exposure. Balls et al. (1996) investigated the influences of microclimate on ozone visible injury in clover, *Trifolium subterraneum* using artificial neural networks. Vapour pressure deficit and light were very strong influences on the extent of ozone injury. These studies demonstrate the importance of other environmental factors in controlling the degree of visible injury, which in turn indicates the difficulty of interpreting visible injury in the field where there are so many factors involved, factors such as genetic diversity within and between populations and greater variation in light, water supply and nutrition. Therefore we consider it is essential to understand the causes of variation in symptoms in order to provide a basis for investigating and understanding the ecological effects of ozone.

The genetic structure of populations has not usually been taken into account when assessing ozone injury even though there are examples that indicate its potential importance. Trembling aspen (*Populus tremuloides*), for example, has ozone-sensitive and resistant genotypes, and the population ozone resistance is related to

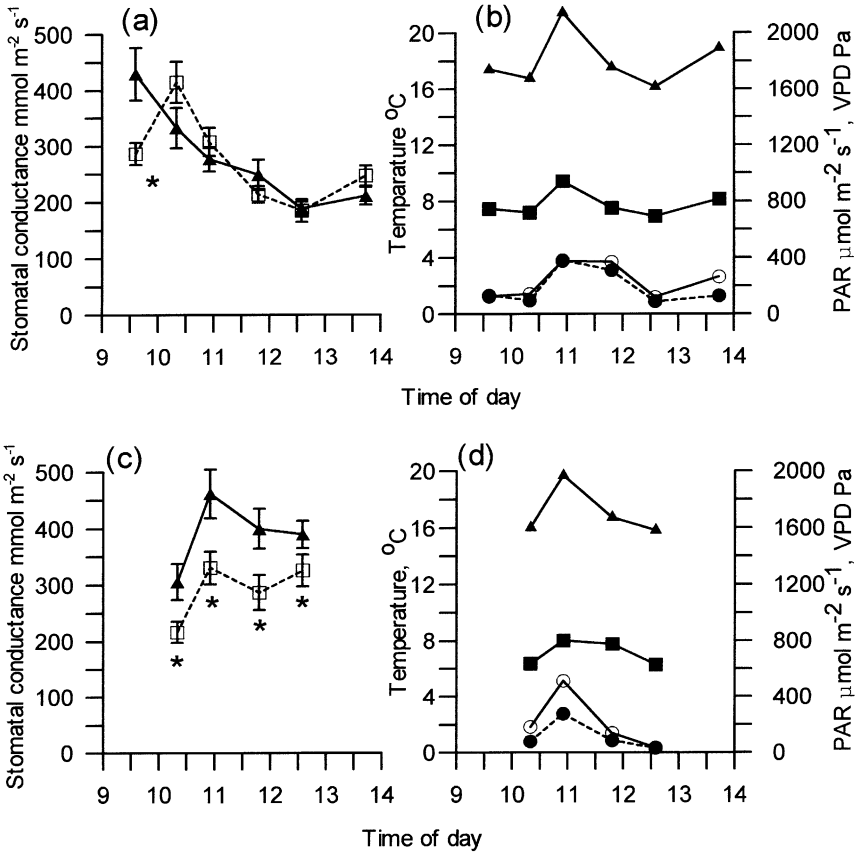

Fig. 7. Mean ($n=5$, ± 1 standard error) stomatal conductance (mmol m^{-2} s^{-1}) of upper and lowermost leaves of coneflower, temperature (°C), PAR (µmol m^{-2} s^{-1}) and vapour pressure deficit (Pa) at Clingman's Dome, 24 July 2000. Graphs (a) and (c) plants on- and off-trail respectively, ▲ = conductance of upper leaves, □ = lower leaves. Graphs (b) and (d) environmental conditions on- and off-trail respectively: ▲ = temperature; ■ = vapour pressure deficit; ○ ● = mean PAR at upper and lower leaves respectively, 30 min preceding reading. ★ = significant difference, $P<0.05$.

the local ozone climate (Berrang et al., 1986, 1989, 1991; Karnosky et al., 1998). In this case a lack of symptoms does not necessarily mean that there is no effect of ozone; the population may be resistant. Aspen also produces large populations that may consist of a single genet, so assessing injury in this species needs great care and knowledge of the clonal structure of populations. However, for most species the degree of genetic variation within populations is not known. It can only be determined conveniently by fingerprinting, using isozymes or DNA. Coneflower proved to be different in genetic structure at the two sites from which it was sampled. At Clingman's Dome the off-trail plants that were sampled from an area of around 20–30 m^2, were all referable to the same genet. However, only 40% of the plants of this genet were injured so environmental factors must have played a vital part in determining ozone flux, sensitivity or symptom expression. At the edge of the trail, where injury was greater and there was a significantly greater per cent (100% in 2001) of injured individuals, the plants were referable to two different, but very closely related genets so the difference in injury between on- and off-trail plants may have had a genetic component. This can only be resolved by transplanting cloned material into the same environment. As a follow-up to the fingerprinting of the Clingman's Dome plants, in 2001 it was thought that one way of estimating the environmental component would be to fingerprint adjacent injured and non-injured plants. If pairs were ramets of the same genet and they showed different degrees of injury, then this would give a measure of the environmental effect. Results were as expected at Clingman's Dome, confirming the previous year's data, but at Purchase Knob the genetic structure was different because all the individuals were very clearly different from each other. Further progress in estimating the environmental and genetic components of variation in injury depends on the transplant experiments that are underway in GSMP and at Newcastle.

It was necessary to measure ozone profiles in canopies because there appear to be no data available for small populations of wild, herbaceous species and no models can currently predict concentrations with enough reliability. As expected, the ozone inside canopies was variable, probably due to differences in canopy structure, stomatal conductance and turbulence, as illustrated in

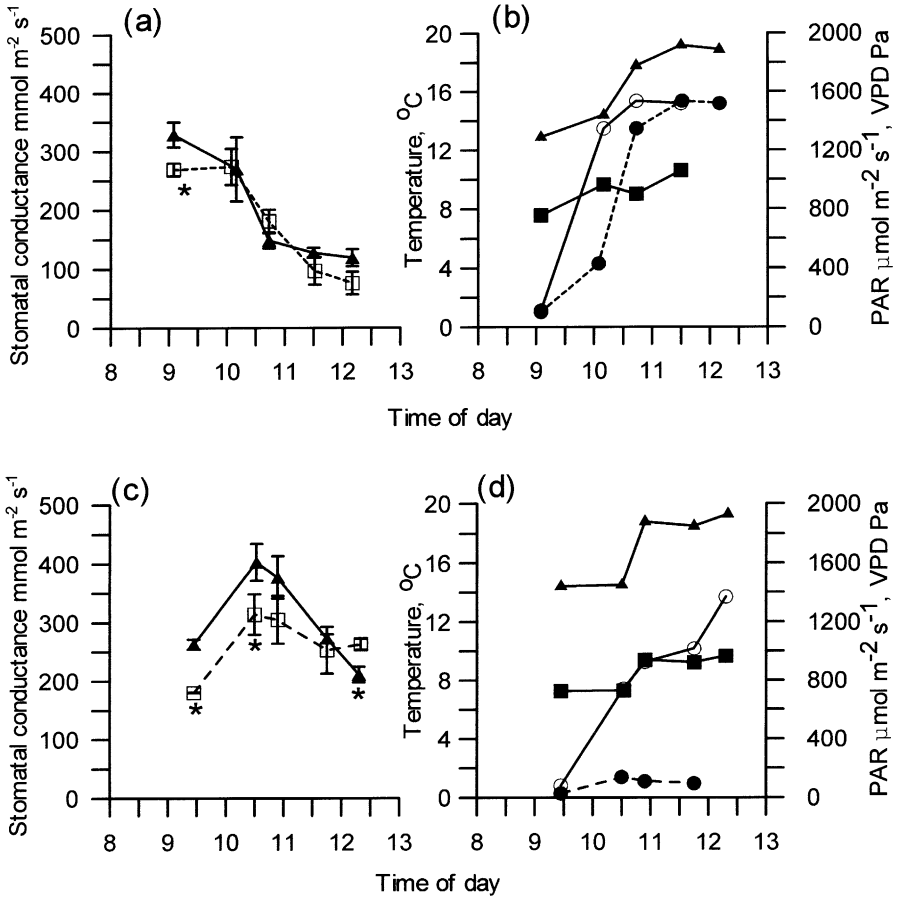

Fig. 8. Mean ($n=5$, ±1 standard error) stomatal conductance (mmol m^{-2} s^{-1}) of upper and lowermost leaves of coneflower, temperature (°C), PAR (µmol m^{-2} s^{-1}) and vapour pressure deficit (Pa) at Clingman's Dome, 20 July 2000. Graphs (a) and (c) plants on- and off-trail respectively, ▲ = conductance of upper leaves, □ = lower leaves. Graphs (b) and (d) environmental conditions on- and off-trail respectively: ▲ = temperature; ■ = vapour pressure deficit; ○ ● = mean PAR at upper and lower leaves respectively, 30 min preceding reading. ★ = significant difference, $P<0.05$.

Figs. 5 and 6. However, there were consistent patterns that help interpret the variation in injury observed in GSMP. The main fact to emerge is that a high per cent of the ozone penetrates into canopies, especially at the edges. The data in Fig. 3 show that it is unlikely that there was sufficient reduction in ozone inside the canopy to account for the abrupt difference in injury between on- and off-trail plants at Clingman's Dome. Figs. 5 and 6 indicate that short-term vertical penetration of ozone into canopies was very variable, and this may help account for differences between populations, but longer term comparisons under a range of conditions are needed. Unfortunately, it was not possible to record air movement but there is little doubt that variation in this factor has a major effect on ozone profiles so future work will focus on the role of wind speed, leaf area index and the various conductances in causing differences in ozone profiles.

Ozone uptake by leaves is governed mainly by atmospheric, boundary layer and stomatal conductances. In the field, stomatal conductance is very variable from place to place and time to time (Jones, 1992), but the

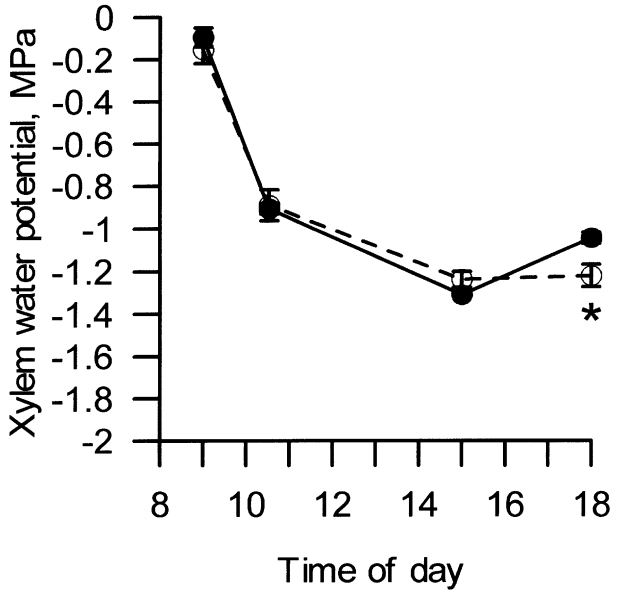

Fig. 9. Xylem water potentials (MPa) in coneflower stems growing at Clingman's Dome, 20 July 2000. ■ = plants on-trail, ○ = plants off-trail. ★ = significant difference, $P<0.05$.

data show that conductance of leaves off-trail or inside populations was usually about the same or higher than at the edges. This means that, other things being equal, ozone uptake inside a stand should be about the same or higher than at the edges. This supports the idea that the difference in injury between on- and off-trail plants was not due to a difference in ozone flux, and conversely points at some other factor being involved. Light may be the important component in this case. It plays a vital part in controlling stomatal conductance but perhaps more important in the case of coneflower, high light is necessary for the formation of anthocyanins (Craker and Wetherbee, 1973; Rabino and Mancinelli, 1986; Mancinelli, 1990; Cone et al., 1993), the pigments that produce the red mottle on coneflower leaves. Therefore it is plausible that the great difference in light within populations (Figs. 3–6) is the main factor determining variation in symptoms in coneflower. If reduction in light does control production of visible symptoms, then it raises the question of whether leaves that are off-trail, with no symptoms were biochemically just as injured as those with anthocyanin mottle. This question can be answered only by controlled fumigation.

5. Conclusions

Overall, the field measurements provided evidence that some coneflower populations consist of very few genets whereas others are genetically very diverse. This complicates the interpretation of injury scores and makes a case for thorough investigation of a species before it is used as a bioindicator. The importance of environment in determining visible symptom expression was demonstrated by the off-trail population at Clingman's Dome, which consisted of a single genet but in which only 40% of individual ramets showed injury. However, the difference between on- and off-trail plants may have had a genetic component as well as an environmental one. Progress in quantifying the effects of genetic and environmental effects depends on comparative experiments using DNA fingerprinted clones in common gardens or open top chambers. Although only a few ozone profiles were recorded under a limited range of conditions they provided evidence that the abrupt variation in symptoms such as that on- and off-trail at Clingman's Dome was unlikely to be caused by lower in-canopy ozone concentrations or lower flux. However, the effects of wind and the magnitude and role of differences in atmospheric and boundary layer conductances need clarification. The reduction in light within canopies may be one of the main factors causing variation in anthocyanin mottle. The role of light is open to relatively easy experimental manipulation.

Acknowledgements

Partial support for this project was provided by funds from the National Geographic Society (project # 6617-99). The authors wish to thank Jim Renfro of the National Parks Service, Efrem Robbins and Kent Burkey for their assistance.

References

Balls, G.R., Palmer-Brown, D., Sanders, G.E., 1996. Investigating microclimatic influences on ozone injury in clover (*Trifolium subterraneum*) using artificial neural networks. New Phytologist 132, 271–280.

Berrang, P., Karnosky, D.F., Bennett, J.P., 1989. Natural selection for ozone tolerance in *Populus tremuloides*: field verification. Canadian Journal of Forest Research 19, 519–522.

Berrang, P., Karnosky, D.F., Bennett, J.P., 1991. Natural selection for ozone tolerance in *Populus tremuloides*: an evaluation of nationwide trends. Canadian Journal of Forest Research 21, 1091–1097.

Berrang, P., Karnosky, D.F., Mickler, R.A., Bennett, J.P., 1986. Natural selection for ozone tolerance in *Populus tremuloides*. Canadian Journal of Forest Research 16, 1214–1216.

Cape, J.N., Unsworth, M.H., 1988. Deposition, uptake, residence of pollutants. In: Schulte-Hostede, S., Darrell, N.M, Blank, L.W., Wellburn, A.R. (Eds.), Air Pollution and Plant Metabolism. Elsevier Applied Science, London, pp. 1–18.

Chappelka, A., Renfro, J., Somers, G., Nash, B., 1997. Evaluation of ozone injury on foliage of black cherry (*Prunus serotina*) and tall milkweed (*Asclepias exaltata*) in Great Smoky Mountains National Park. Environmental Pollution 95, 13–18.

Cone, K.C., Cocciolone, S.M., Moehlenkamp, C.A., Weber, T., Drummond, B.J., Tagliani, L.A., Bowen, B.A., Perrot, G.H., 1993. Role of the regulatory gene pl in the photocontrol of maize anthocyanin pigmentation. Plant Cell 5, 1807–1816.

Craker, L.E., Wetherbee, P.J., 1973. Ethylene, light and anthocyanin synthesis. Plant Physiology 51, 436–438.

Davison, A.W., Barnes, J.D., 1998. Effects of ozone on wild plants. New Phytologist 139, 135–151.

Davison, A.W., Reiling, K., 1995. A rapid change in ozone resistance of *Plantago major* after summers with high ozone concentrations. New Phytologist 131, 227–343.

Enders, G., 1992. Deposition of ozone to a mature spruce forest—measurements and comparison to models. Environmental Pollution 75, 61–67.

Fontan, J.A., Minga, A., Lopez, A., Druilhet, A., 1992. Vertical ozone profiles in a pine forest. Atmospheric Environment 26A, 863–869.

Fredricksen, T.S., Joyce, B.J., Skelly, J.M., Steiner, K.C., Kolb, T.E., Kouterick, K.B., Savage, J.E., Snyder, K.R., 1995. Physiology, morphology and ozone uptake of leaves of black cherry seedlings, saplings and canopy trees. Environmental Pollution 89, 273–283.

Jones, H.G., 1992. Plants and Microclimate: A Quantitative Approach to Environmental Physiology. Cambridge University Press, Cambridge.

Joss, U., Graber, W.K., 1996. Profiles and simulated exchange of H_2O, O_3, NO_2 between the atmosphere and the HartX Scots pine plantation. Theoretical and Applied Climatology 53, 157–172.

Karnosky, D.F., Podila, G.K., Gagnon, Z., Pechter, P., Akkapeddi, A., Sheng, Y., Riemenschneider, D.E., Coleman, M.D., Dickson, R.E., Isebrands, J.G., 1998. Genetic control of responses to interacting ozone and CO_2 in *Populus tremuloides*. Chemosphere 36, 807–812.

Krupa, S.V., Manning, W.J., Nosal, M., 1993. Use of tobacco cultivars as biological indicators of ambient ozone pollution: an analysis

of exposure-response relationships. Environmental Pollution 81, 137–146.

Lorenzini, G., Nali, C., 1995. Analysis of vertical ozone and nitrogen oxides profiles in a *Prunus cerasus* canopy. International Journal of Biometeorology 39, 1–4.

Mancinelli, A.L., 1990. Interaction between light quality and light quantity in the photoregulation of anthocyanin production. Plant Physiology 92, 1191–1195.

Neufeld, H.S., Renfro, J.R., Hacker, W.D., Silsbee, D., 1992. Ozone in Great Smoky Mountains National Park: dynamics and effects on plants. In: Berglund, R.D. (Ed.), Tropospheric Ozone and the Environment II. Air and Waste Management Association, Pittsburgh, PA, pp. 594–617.

Rabino, I., Mancinelli, A.L., 1986. Light, temperature, and anthocyanin production. Plant Physiology 81, 922–924.

Samuelson, L.J., Kelly, J.M., 1997. Ozone uptake in *Prunus serotina*, *Acer rubrum* and *Quercus rubra* forest trees of different sizes. New Phytologist 136, 255–264.

Shaver, C.L., Tonnessen, K.A., Maniero, T.G., 1994. Clearing the air at Great Smoky Mountains National Park. Ecological Applications 4, 690–701.

Weising, K., Nybom, H., Wolff, K., Meyer, W., 1995. DNA Fingerprinting in Plants and Fungi. CRC Press, Boca Raton, FL.

Whitfield, C., Davison, A.W., Ashenden, T.W., 1997. Artificial selection and heritability of ozone resistance in two populations of *Plantago major* L. New Phytologist 137, 645–655.

Wolff, K., Morgan-Richards, M., Davison, A.W., 2000. Patterns of molecular genetic variation in *Plantago major* and *P. intermedia* in relation to ozone resistance. New Phytologist 145, 501–509.